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ORIGINAL RESEARCH

The Effect of Dimethyl Fumarate on Cerebral Gray Matter Atrophy in Multiple Sclerosis

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ABSTRACT

Introduction: The objective of this pilot study was to compare cerebral gray matter (GM) atrophy over 1 year in patients starting dimethyl fumarate (DMF) for multiple sclerosis (MS) to that of patients on no disease-modifying treatment (noDMT). DMF is an established therapy for relapsing–remitting (RR) MS.

Methods: We retrospectively analyzed 20 patients with RRMS at the start of DMF [age (mean ± SD) 46.1 ± 10.2 years, Expanded Disability Status Scale (EDSS) score 1.1 ± 1.2, timed 25-foot walk (T25FW) 4.6 ± 0.8 s] and eight patients on noDMT (age 42.5 ± 6.6 years, EDSS 1.7 ± 1.1, T25FW 4.4 ± 0.6 s). Baseline and 1-year 3D T1-weighted 3T MRI was processed with automated pipelines (SIENA, FSL-FIRST) to assess percentage whole brain volume change (PBVC) and deep GM (DGM) atrophy. Group differences were assessed by analysis of covariance, with time between MRI scans as a covariate.

Results: Over 1 year, the DMF group showed a lower rate of whole brain atrophy than the noDMT group (PBVC: −0.37 ± 0.49% vs. −1.04 ± 0.67%, $p = 0.005$). The DMF group also had less change in putamen volume (−0.06 ± 0.22 vs. −0.32 ± 0.28 ml, $p = 0.02$). There were no significant on-study differences between groups in caudate, globus pallidus, thalamus, total DGM volume, T2 lesion volume, EDSS, or T25FW (all $p > 0.20$).

Conclusions: These results suggest a treatment effect of DMF on GM atrophy appearing at 1 year after starting therapy. However, due to the retrospective study design and sample size, these findings should be considered preliminary, and require confirmation in future investigations.

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INTRODUCTION

Conventional brain MRI measures of multiple sclerosis (MS)-related disease activity, such as the number of gadolinium (Gad)-enhancing lesions and new or enlarging T2-weighted lesions, are standard supportive outcome measures in clinical trials of disease-modifying therapies (DMTs) [1–4]; however, additional measures have been shown to provide complementary information on the efficacy of DMTs [1, 5, 6]. Brain atrophy has become increasingly important due to its core role in the pathophysiology of MS, correlations with clinical dysfunction, and the technological post-processing advances allowing a continuing increase in the sophistication of MRI quantification methods [1, 5–19]. In addition, the use of higher-field (i.e., 3T) MRI scanners provides the potential for increased accuracy and reliability in the assessment of lesions and atrophy [9, 10, 20]. Brain atrophy was once thought to be predominantly the result of white matter (WM) volume loss, but it is now widely accepted that gray matter (GM) bears the brunt of such atrophy [13]. Furthermore, several studies have indicated that GM atrophy may be a more reliable marker of treatment effects than WM atrophy, as WM volume is prone to fluctuations [21]; this is partly on the basis of pseudoatrophy in the first few months after the initiation of DMTs [22, 23], or transient increases in WM volume due to inflammation [24]. GM atrophy occurs early in the MS disease course, is related to immunologic changes, physical disability, cognitive dysfunction, depression, and quality of life, and can predict long-term clinical changes [1, 5, 7, 8, 10, 11, 13, 15, 17, 18, 25–33]. Thus, assessing therapeutic effects on GM atrophy is an attractive strategy for further exploration [12].

Phase III MS clinical trials have shown that several DMTs partially, but significantly, reduce the rate of whole brain atrophy when compared to placebo treatment [6, 13, 34–44]; however, assessments of such effects on cerebral GM atrophy are largely limited to smaller post hoc or phase IV studies [12, 18, 19, 23, 45–47].

Dimethyl fumarate (DMF) is an orally administered agent approved for the treatment of relapsing–remitting (RR) MS. Its proposed mechanism of action involves the activation of the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway and Nrf2 independent pathways to increase resistance against oxidative stress, potentially providing both anti-inflammatory and neuroprotective effects [48, 49]. DMF has been shown to significantly reduce relapse rates, progression of physical disability, and the accumulation of brain MRI lesions in patients with RRMS when compared to placebo [37, 38, 50–54]. The pivotal phase III trials have also shown that DMF therapy significantly reduces the rate of whole brain volume loss compared to placebo [37, 38]. However, no published study to date has assessed the impact of DMF on GM damage, such as the progression of GM atrophy. The objective of this pilot study was to compare the 1-year rate of cerebral GM atrophy in patients with RRMS after the start of DMF, as compared to untreated patients, in a "real-world" clinical care setting.

METHODS

Subjects

Baseline demographic and clinical characteristics of all subjects are presented in

Table 1. We retrospectively analyzed 20 consecutive patients with RRMS who were newly starting oral DMF therapy (120 mg twice a day for 1 week, then 240 mg twice a day) and eight patients (six RRMS, two clinically isolated syndrome) on no disease-modifying therapy (noDMT) for 1 year. All subjects were identified by chart review using the following inclusion criteria: age 18–60 years, Expanded Disability Status Scale (EDSS) score of 0–5 at baseline, no corticosteroid use within 30 days of MRI, and the availability of baseline and 1-year follow-up brain 3T MRI. Prior to starting DMF, patients had been previously treated with the following DMTs: glatiramer acetate (GA; $n = 8$), intramuscular interferon beta-1a (IFNβ-1a, once a week; $n = 7$), subcutaneous interferon beta-1a (thrice weekly; $n = 4$), interferon beta-1b ($n = 2$), and natalizumab ($n = 2$). Patients in the noDMT group had no prior history of exposure to any DMT. All patients underwent clinical evaluations, including EDSS scoring and timed 25-foot walk (T25FW), within 3 months of MRI by an MS specialist neurologist at our institution. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2013. Informed consent was not required due to the retrospective nature of the study.

Table 1 Baseline demographic, clinical, and MRI data

	DMF	noDMT	*p* value
Number of patients with multiple sclerosis	20	8	–
Age (years)	46.1 ± 10.2 (26.9–60.7)	42.5 ± 6.6 (30.7–50.8)	0.47‡
Women, no. (%)	13 (65%)	8 (100%)	0.07†
Disease duration (years)*	12.4 ± 8.0 (1.3–35.7)	6.7 ± 6.8 (0.2–17.0)	0.15‡
Expanded Disability Status Scale score	1.1 ± 1.2 (0–3.5)	1.7 ± 1.1 (0–3.0)	0.22‡
Timed 25-foot walk (s)	4.6 ± 0.8 (3.3–6.7)	4.4 ± 0.6 (3.5–5.2)	0.68‡
Time on DMF vs. baseline MRI (months)**	2.0 ± 2.8 (−2 to 6)	–	–
Thalamus volume (ml)	15.2 ± 1.7 (13.0–18.3)	14.5 ± 0.8 (13.4–15.6)	0.33‡
Caudate volume (ml)	6.4 ± 0.8 (5.1–8.0)	6.5 ± 1.0 (5.0–8.1)	0.90‡
Putamen volume (ml)	9.4 ± 1.0 (7.6–11.6)	9.4 ± 1.2 (7.9–11.1)	0.98‡
Globus pallidus volume (ml)	3.5 ± 0.4 (2.8–4.4)	3.3 ± 0.3 (2.9–3.7)	0.28‡
Total DGM volume (ml)	34.6 ± 3.3 (29.6–42.1)	33.7 ± 3.0 (29.7–38.3)	0.53‡
Cerebral T2 lesion volume (ml)	8.6 ± 11.6 (0.4–46.5)	2.7 ± 1.7 (0.6–6.2)	0.28‡
Cerebral # of gadolinium enhancing lesions	0.9 ± 1.3 (0–5)	0.3 ± 0.5 (0–1)	0.35§

Data are shown as mean ± standard deviation (range), except as otherwise indicated; * years from first symptoms; *DMF* dimethyl fumarate; *noDMT* no disease-modifying therapy; total cerebral deep gray matter (*DGM*) = thalamus + caudate + putamen + globus pallidus; ** negative number indicates that therapy was started after baseline MRI; † Fisher's exact test; ‡ Exact Wilcoxon test; § Exact Jonckheere–Terpstra test

MRI Acquisition

All subjects underwent 3T brain MRI using the same scanner (3T Skyra; Siemens Medical Solutions, Malvern, PA, USA) and similar acquisition protocol. We chose 3T for this study based on the higher diagnostic sensitivity it has shown in the detection of brain lesions and atrophy in MS [9, 10, 20]. The relevant images included (1) sagittal 3D T1-weighted magnetization-prepared rapid acquisition gradient echo (MPRAGE) images (TR/TE: 2300/2.96 ms for all except one patient with TR/TE: 1800/2.84 ms; voxel size: $1 \times 1 \times 1$ mm) and (2) axial 2D T2-weighted fluid-attenuated inversion-recovery (FLAIR) images (TR/TE: 9000/81 ms; voxel size: $0.6875 \times 0.6875 \times 3$ mm for all except one patient with voxel size: $0.4297 \times 0.4297 \times 3$ mm). In addition, axial 2D T1-weighted spin-echo post-contrast images were acquired 5–7 min after a single dose of intravenous Gad (TR/TE: 474–916/88–200 ms; voxel size: $0.4297 \times 0.4297 \times 3$ mm for all except a patient with $0.8594 \times 0.8594 \times 3$ mm). For the DMF group, baseline MRI was conducted within (mean ± SD) 2.0 ± 2.8 months (range 6 months prior to 2 months post) of the start of DMF. Follow-up MRI for all subjects was obtained at a similar interval between groups within a range of 9–15 months after baseline MRI (DMF group: 12.2 ± 1.2 months; noDMT group: 11.8 ± 2.1 months; $p = 0.59$).

MRI Analysis

Pre-processing

All image pre-processing was performed using Jim software (version 7.0; Xinapse Systems Ltd., West Bergholt, UK, http://www.xinapse.com/). The raw sagittal images did not yield adequate segmentation, particularly of the intracranial volume cavity (data not shown). Based on optimization work, all original DICOM images were first converted to a Neuroimaging Informatics Technology Initiative (NIfTI) format, and their native sagittal orientation was converted to axial. Axial slices were then extracted from each scan starting at the first slice showing the top of the head and continuing to the foramen magnum. This provided whole brain coverage in all subjects.

Global Cerebral Atrophy

The MPRAGE images were applied to an automated pipeline, the Structural Image Evaluation using Normalization of Atrophy (SIENA v. 5.0; Analysis Group, Oxford, UK, http://fsl.fmrib.ox.ac.uk), to assess the intra-subject percentage whole brain volume change (PBVC) between baseline and follow-up scans (Fig. 1). The use of SIENA to assess longitudinal whole brain atrophy is well established [14, 27, 38, 55]. To assess the reliability of this method with our acquisitions, three randomly chosen patients with MS and three healthy controls underwent an MPRAGE scan followed by a re-scan on the same day. Thus, two scans were acquired from each subject, where the subject was removed from the scanner for a few minutes between scans, and was repositioned and re-scanned by the MRI technologist. The MPRAGE acquisitions were performed with the same scanner platform and acquisition protocol as that employed in the present study. In these six scan–re-scan subjects, the PBVC was $-0.080 \pm 0.26\%$, indicating high reliability.

Cerebral Deep Gray Matter (DGM) Atrophy

MPRAGE images were applied to an automated model-based segmentation tool, the Oxford Centre for Functional MRI of the Brain

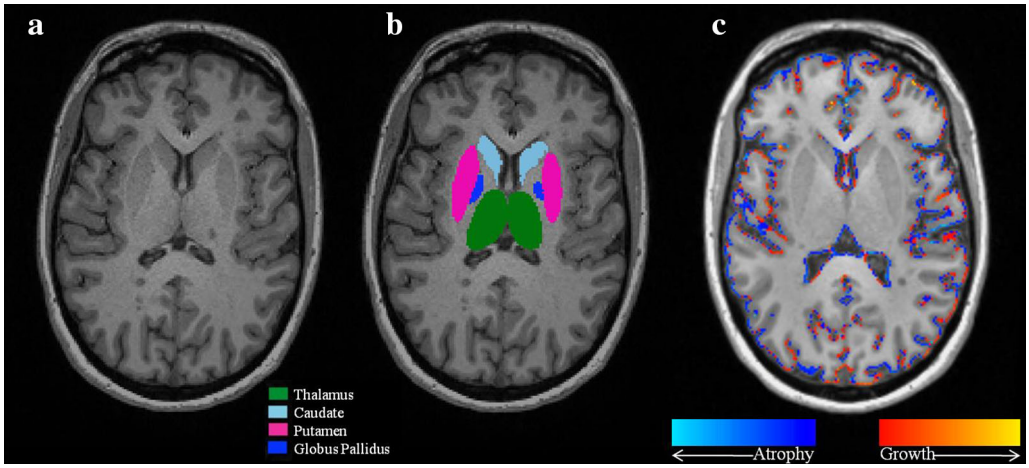

Fig. 1 Brain volume measurement techniques: regional and global metrics. A raw 3D T1-weighted magnetization-prepared rapid acquisition gradient echo (MPRAGE) image that has been re-sampled to axial (**a**) and an example of an FSL/FIRST [FMRIB (Oxford Centre for Functional MRI of the Brain) Software Library Integrated Registration and Segmentation Tool] *deep gray* matter segmentation (**b**) of the same MRI slice. In the present study, we utilized the FSL/FIRST outputs to assess the volume of the thalamus, caudate, putamen, and globus pallidus (and their sum = total deep gray matter). In addition, we employed SIENA (Structural Image Evaluation using Normalization of Atrophy) to create a difference map in each subject to assess the within-subject change in whole brain volume from baseline to 1-year follow-up. One anatomic slice from the SIENA output is shown (**c**) for a subject. The colors on the image indicate the brain parenchymal change between baseline and follow-up, based on shifts of the brain boundary. *Lighter blue colors* indicate brain tissue volume loss over time (atrophy), whereas *yellow colors* indicate tissue volume increase. In this case, the patient experienced whole brain atrophy-percentage whole brain volume change of −1.69%. The images are from a 31-year-old woman with multiple sclerosis from the no disease-modifying therapy group, disease duration 2.4 years, baseline Expanded Disability Status Scale score 2, and baseline timed 25-foot walk 5.2 s

Software Library Integrated Registration and Segmentation Tool (FSL-FIRST, version 5.0; Analysis Group, Oxford, UK, http://fsl.fmrib.ox.ac.uk), to determine whole-structure volume of the putamen, caudate, globus pallidus, and thalamus. The total DGM was the sum of these four structures (Fig. 1). The use of FSL-FIRST to determine the volume of sub-cortical structures is well established [9, 26, 56]. Our previous experience with this method shows high scan–re-scan reliability, with an average intra-class correlation coefficient of 0.99 [9].

Lesion Analysis

Whole brain T2 hyperintense lesion volume (T2LV) analysis was performed using a semi-automated method based on expert tracing from the FLAIR images using Jim software (version 7; Xinapse Systems Ltd., West Bergholt, UK; http://www.xinapse.com), as previously described [20]. The number of cerebral Gad-enhancing lesions was determined from axial 2D T1-weighted post-Gad images by the same observer. Our methods for quantifying lesions have been shown to be highly reliable, as detailed previously [57].

All analysis was conducted in a blinded manner; researchers were unaware of clinical and demographic characteristics. Brain atrophy analysis was conducted by one observer (RC). Lesion analysis was conducted by a single observer (SD) and confirmed by a senior observer (ST).

Statistical Analysis

Baseline demographic and clinical comparisons were conducted using exact Wilcoxon and Jonckheere–Terpstra tests. Cerebral GM atrophy measures were assessed by analysis of covariance with time between scans as a covariate. Lesion comparisons were assessed using Wilcoxon rank sum, Fisher's exact, and van Elteren tests (when adjusting for time between scans). Correlations were assessed using Spearman correlations. A value of $p < 0.05$ was considered statistically significant; $p \geq 0.05$ but <0.10 was considered a trend toward significance.

RESULTS

Baseline Demographic, Clinical, and MRI Comparisons

No significant differences were detected between the DMF and noDMT groups in terms of baseline age, EDSS score, or T25FW (all $p > 0.20$). The DMF and noDMT groups did not differ in terms of baseline T2LV, the number of Gad-enhancing lesions, or DGM volumes (all $p > 0.25$) (Table 1).

On-Study Change in Global Cerebral Atrophy

In comparisons of the change in whole brain volume from baseline to follow-up, the DMF group showed less atrophy, with a lower PBVC, than the noDMT group ($-0.37 \pm 0.49\%$ vs. $-1.04 \pm 0.67\%$, $p = 0.02$; when taking into account the time between baseline and follow-up MRI scans: $p = 0.005$) (Table 2; Fig. 2).

Table 2 Brain volume change on-study: global and regional deep gray matter

	DMF	noDMT	*p* value
Thalamus	-0.10 ± 0.28	-0.04 ± 0.34	0.50
Caudate	-0.08 ± 0.12	-0.01 ± 0.15	0.28
Putamen	-0.06 ± 0.22	-0.32 ± 0.28	0.02*
Globus pallidus	-0.04 ± 0.11	-0.04 ± 0.10	1.00
Total DGM	-0.28 ± 0.42	-0.41 ± 0.80	0.69
PBVC	-0.37 ± 0.49	-1.04 ± 0.67	0.005*

Data represent mean ± standard deviation difference between baseline and 1-year follow-up in ml for all variables except for percent whole brain volume change (PBVC), which shows %; *DMF* dimethyl fumarate; *noDMT* no disease-modifying therapy; total cerebral deep gray matter (DGM) = thalamus + caudate + putamen + globus pallidus; *p* values shown are with time between scans as a covariate; $^{*}p < 0.05$

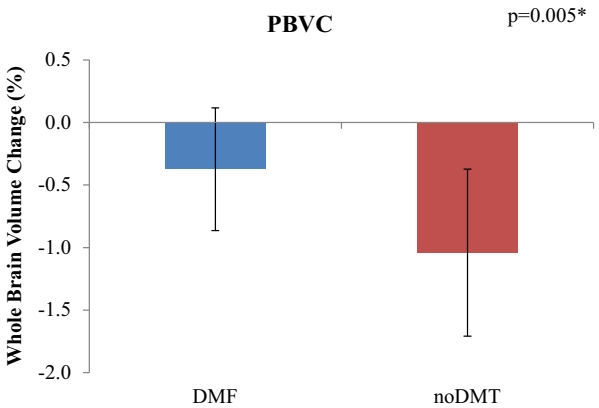

Fig. 2 Brain volume change on-study: whole brain atrophy. Percentage whole brain volume changes (PBVC) from baseline to follow-up were assessed in patients with multiple sclerosis on dimethyl fumarate (DMF) therapy vs. patients on no disease-modifying therapy (noDMT). Means and standard deviation bars are shown. On average, the DMT group had a 64.4% lower rate of whole brain atrophy than the noDMT group ($p = 0.02$; $p = 0.005$, adjusted for MRI interval). $^{*}p < 0.05$

On-Study Change in DGM Atrophy

In comparisons of on-study DGM volume changes, the DMF group showed significantly less atrophy, with smaller putamen volume changes, than the noDMT group (-0.06 ± 0.22 vs. -0.32 ± 0.28 ml, $p = 0.04$). There were no significant on-study differences between groups in caudate, globus pallidus, thalamus, or total DGM volumes (all $p > 0.35$) (Table 2; Fig. 3). Similar results were found when adjusting for the time between baseline and follow-up MRI scans (putamen $p = 0.02$; other volumes $p > 0.25$) (Table 2; Fig. 3).

On-Study Change in Lesions

In terms of on-study T2LV and Gad count change, no significant differences were found between the two groups (both $p > 0.40$). Similar

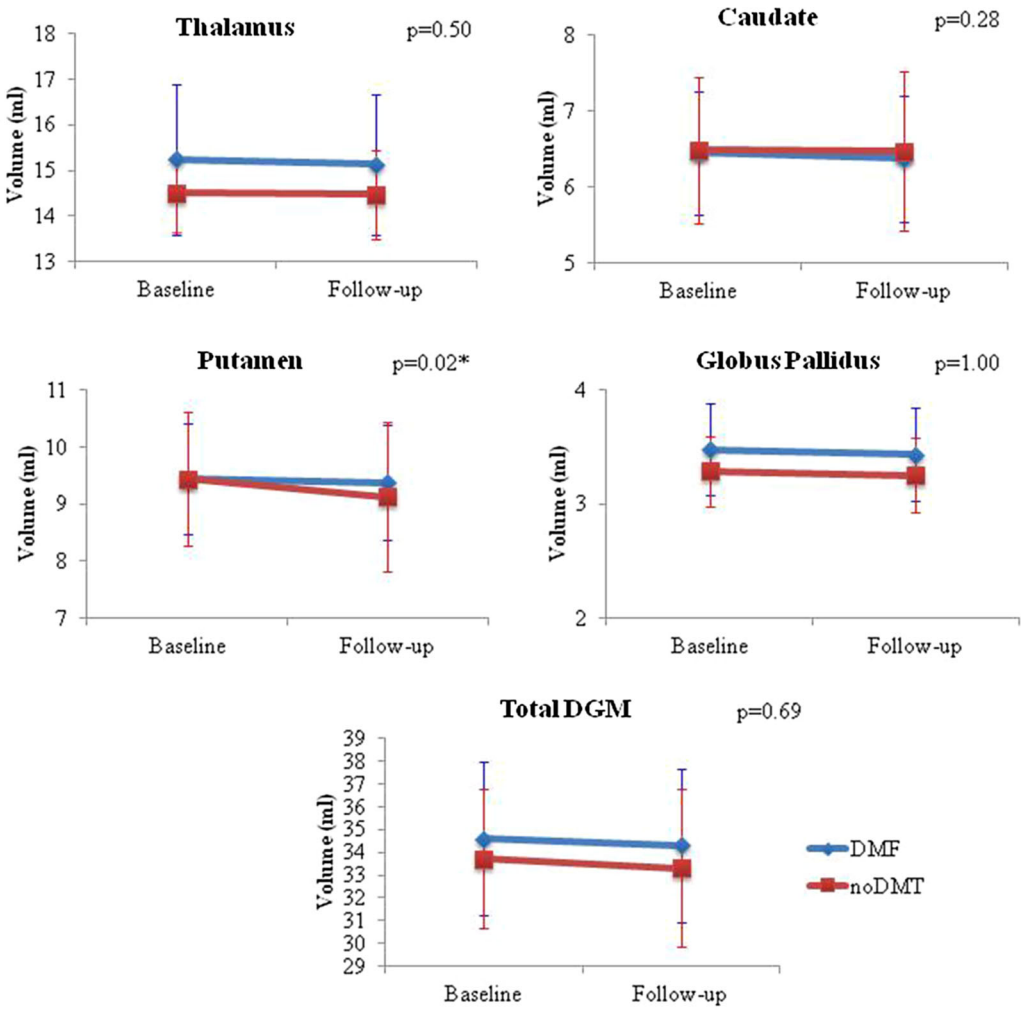

Fig. 3 Brain volume change on-study: regional (*deep gray* matter) atrophy. Changes in cerebral *deep gray* matter (DGM) volume over 1 year in a group of multiple sclerosis (MS) patients on dimethyl fumarate (DMF) therapy (*blue*) in comparison to patients on no disease-modifying therapy (noDMT) (*red*). Means and standard deviations are shown. Total DGM = thalamus + caudate + putamen + globus pallidus. Exact Wilcoxon tests indicated that the putamen volume change differed significantly between the two groups, showing a lower rate of atrophy in the DMF group ($p = 0.02$); p values were calculated with time between scans as a covariate; $^{*}p < 0.05$

results were found when adjusting for scan interval (both $p > 0.30$, van Elteren test). T2LV increased by 0.47 ± 1.25 ml in the DMF group and 0.05 ± 0.47 in the noDMT group. The number of Gad-enhancing lesions decreased by 0.25 ± 1.25 in the DMF group and 0.13 ± 0.35 in the noDMT group.

Additional Statistical Analysis of Treatment Effect Adjusting for Baseline Characteristics

The results did not change appreciably in multiple variable models when controlling for baseline T2LV, number of Gad-enhancing lesions, and age (in addition to the time between scans). The group comparison of on-study PBVC and putamen volume change remained significant in all models ($p < 0.05$). In subset analyses that accounted for the time between scans and were also balanced on age (<52 years, $n = 22$), T2LV (<7.0 ml, $n = 20$), number of Gad-enhancing lesions (≤ 1, $n = 23$), and with all three restrictions ($n = 17$), the group differences in the changes in putamen volume and PBVC remained meaningful in favor of the DMF group. For example, in subset analyses ($n = 17$) with concomitant reduced power, where the two groups were balanced on all three restrictions, the predicted on-study putamen volume decrease was 0.07 ml for DMF and 0.31 ml for noDMT ($p = 0.10$); the predicted PBVC decrease was 0.33 for DMF and 1.05 for noDMT ($p = 0.02$).

On-Study Clinical Changes

When comparing DMF and noDMT groups in terms of on-study clinical relapses, no significant differences were noted; one patient in the noDMT group experienced a relapse (per patient relapse rate 12.5%) in comparison to 2 patients in the DMF group (rate 10%) ($p = 1.0$). In addition, no significant differences were found between groups in terms of on-study changes in EDSS score (DMF group: 0 ± 1.19 vs. noDMT group: -0.56 ± 1.05, $p = 0.22$) or T25FW (DMF group: 0.12 ± 0.85 s vs. noDMT: 0.36 ± 1.36 s, $p = 0.82$). Similar results were found when adjusting for time between baseline and follow-up examination ($p = 0.23$ and $p = 0.43$ for on-study changes in EDSS score and T25FW, respectively).

Correlation Analysis

When assessing relationships between DGM volume and lesions in all subjects, a significant correlation was found between total DGM volume and T2LV at baseline (Spearman $r = -0.45$, $p = 0.02$); however, no significant correlations were found between the on-study changes in total DGM volume and on-study changes in T2LV, baseline T2LV, or baseline age ($p > 0.35$). In addition, no significant correlations were found between baseline DGM volume and T25FW or EDSS score (all $p > 0.05$; baseline total DGM volume vs. baseline EDSS showed a trend toward significance: $r = -0.32$, $p = 0.09$). Similarly, no significant correlations were detected between baseline T2LV and baseline T25FW or EDSS score (both $p > 0.85$). Regarding the ability of baseline variables to predict on-study change in whole brain volume, no significant correlations were detected when comparing baseline T2LV or age to PBVC (all $p > 0.80$).

DISCUSSION

The purpose of this pilot study was to compare the rate of cerebral GM atrophy in patients with RRMS recently started on DMF vs. untreated

patients. Our results show that the DMF group experienced a lower rate of whole brain atrophy than the noDMT group. Additionally, the DMF group had a lower rate of putamen atrophy. No significant on-study differences were detected between groups in caudate, globus pallidus, thalamus, total DGM volume, T2 lesion volume, or clinical measures. These exploratory results provide novel information both in the suggestion of a treatment effect on brain atrophy in the first year after starting DMF and in the demonstration of such effects on GM atrophy. However, these findings should be considered preliminary due to the small sample size and the non-randomized study design.

The results of the present study suggest a treatment effect on brain atrophy appearing in the first year after the start of DMF therapy. In the two pivotal phase III RRMS trials of DMF, a dosage of 240 mg twice a day showed a partial but significant effect at 2 years in limiting the rate of whole brain atrophy compared to placebo. In the DEFINE study, DMF showed a statistically significant 21% lower rate of atrophy from 0 to 24 months, and a significant 30% lower rate from 6 to 24 months of the study; however, a 1-year time point MRI scan was not performed [37]. In the CONFIRM study, DMF showed a statistically significant 32% lower rate of atrophy between years 1 and 2, although no significant treatment effect was detected in the first year [38]. With regard to the other approved DMTs for RRMS with effects on limiting brain atrophy apparent in the first year, fingolimod has shown an effect on whole brain atrophy in two pivotal phase III studies, apparent at 6 months, as compared to placebo [43, 44]. Patients on teriflunomide experienced significant reductions in brain volume change in comparison to placebo at 1 and 2 years [42]. Additionally, one of the pivotal phase III studies of alemtuzumab showed a lower rate of whole brain atrophy in comparison to patients on IFNβ-1a at 1 year [58]; the treatment effect was also apparent over 2 years [40, 41]. Similarly, patients on daclizumab experienced significant reductions in the rate of whole brain volume loss compared to patients on IFNβ-1a in both the first and second year of treatment [59]. The demonstration of such early treatment effects on brain atrophy has otherwise typically required 2 years in MS clinical trials [13, 19, 34]. For instance, patients on intramuscular or subcutaneous IFNβ-1a showed a significant reduction in whole brain atrophy in comparison to patients on placebo after 2 years, but not in the first year [6, 35]. Likewise, patients on GA demonstrated a significant reduction in the rate of whole brain volume loss compared to placebo only in the second 9 months after the start of therapy [36]. Patients on natalizumab showed a significant reduction in the rate of whole brain atrophy compared to placebo in the second but not first year of therapy [39]. The lack of an early treatment effect in these studies has been attributed to two main factors. First, pseudoatrophy may occur in the initial few months after starting DMTs, due to a net loss of edema fluid and inflammatory cells from the brain to the systemic circulation [13, 21, 34]. Second, a time delay may exist for some DMTs, for which the downstream results of their immunomodulatory benefits on ultimate tissue loss may require several months to achieve [6].

To further understand the effect of DMF on brain atrophy, the present study focused on its impact on GM atrophy. Analysis of DGM structures showed a lower rate of atrophy of the putamen over 1 year in the DMF vs. the noDMT group. A limited number of previous

studies have assessed the impact of other DMTs on cerebral GM [19, 60]. For instance, studies have shown that the effect of IFNβ-1a on brain atrophy may be driven primarily by its impact on GM atrophy [23, 45, 46]. In addition, emerging studies have suggested that natalizumab limits cerebral GM atrophy or other structural changes [12, 47]. A treatment effect on GM atrophy would have potentially high relevance for patients. GM atrophy occurs early in MS, is clinically relevant, and worsens as the disease progresses [1, 5, 7, 10, 13, 15, 17, 18, 24–33, 57, 61, 62]. Atrophy in the DGM can be detected during the first clinical stages of MS, even as early as the first few years after symptom onset [26].

There are several possible mechanisms by which the MS disease process leads to DGM atrophy. Considering these will help us understand how DMF may exert its therapeutic effect. First, the DGM is highly interconnected with the rest of the brain [63–65], and thus is quite vulnerable to the "dying back" (Wallerian degeneration) effects of neuronal loss following MS-related WM demyelination and axonal transection [65–68]. This would be consistent with the present findings, showing a significant inverse correlation between T2LV and total DGM volume. Second, the DGM is thought to be a site of excessive iron deposition in patients with MS, which has been linked to physical disability, cognitive dysfunction, and brain atrophy [69, 70]. While deposition of iron may represent purely an epiphenomena of neurodegeneration, one must consider the possibility that iron-mediated (or other forms of) oxidative stress and lipid peroxidation may target the DGM [71]. The third aspect to consider is direct injury to the DGM by the presence of demyelinating foci (lesions); 7T MRI has been pivotal in showing an abundance of such lesions, which are linked to physical disability, progressive disease, and cortical lesions [72]. These are histologically characterized by demyelination, inflammation, and axonal damage [64]. The fourth theory is based on the proximity of the DGM to the ventricular CSF, in which activated lymphocytes are thought to enter via the choroid plexus [73]. DMF has the potential to confer its protective effect on brain atrophy by targeting all four of these proposed mechanisms. This includes its neuroprotective effects via the Nrf2 antioxidant pathway [48], and a wide range of immune effects, such as decreasing T-cells [74], increasing the number of circulating regulatory B-cells [75], exerting immunomodulatory effects on T-cell subsets and antigen-presenting cells [76], downregulating pro-inflammatory cytokines [77], and upregulating the anti-inflammatory cytokine interleukin 10 [78].

Our study has several potential limitations and internal inconsistencies that must be considered. For example, the present study did not show a treatment effect on MRI-defined lesions. This contradicts the findings of phase III clinical trials that have indicated the ability of DMF to limit the appearance of new or enlarging T2 and Gad lesions [37, 38]. Additionally, we noted a lack of treatment effect on clinical measures including relapse rate, EDSS score, and T25FW. Such inconsistencies may be a result of the small sample size, non-randomized comparison, and the limited duration of the study, given that previous trials assessed treatment effects over 2 years as opposed to 1 year. Our results demonstrated a significant effect on putamen volume change, but not on any other DGM structure or total DGM. This may be due to the fact that DGM nuclei are small structures with complex shapes and sizes, making them

susceptible to measurement biases. It may also suggest that a longer treatment period would yield volume changes more readily detected by our methods of quantification. In addition, the patients in the DMF arm had been previously treated with other DMTs, and it is not known whether long-term effects of the previous agents influenced our results, even after these treatments were stopped. Lastly, the retrospective design of the present study may have had an impact on our findings. Despite these factors, however, our findings could serve as an impetus for future studies to assess the effects of DMF treatment on cerebral GM in larger prospective controlled studies.

CONCLUSIONS

The results of this study suggest a treatment effect of DMF on GM atrophy appearing 1 year after starting therapy. However, due to the retrospective study design and sample size, these findings should be considered preliminary, and require confirmation in future investigations.

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Compliance with Ethics Guidelines. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2013. Informed consent was not required due to the retrospective nature of the study.

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